

## **Aspirator Gun for High-Throughput Mosquito Bioassays**

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## OPERATIONAL NOTE

### ASPIRATOR GUN FOR HIGH-THROUGHPUT MOSQUITO BIOASSAYS

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**ABSTRACT.** We describe an innovative aspirator gun designed to transfer individual anesthetized mosquitoes directly into glass bioassay tubes. The gun has been used for thousands of transfers with extremely low associated mortality and is the central component of a high-throughput bioassay system. The gun is constructed using readily obtainable materials and can be modified for a range of insects.

**KEY WORDS** Mosquito, arthropod, insect, aspirator, suction

Aspirators are indispensable tools for conducting field and laboratory research with mosquitoes (Peterson 1953, Fergusson 1982, Arnold 1994, Tang 1996, Toth 2000). Aspirators were originally developed to use mouth suction (Psota 1916); however, aspirators using powered suction, such as with bulbs or bellows (Wishart 1930, Brophy et al. 1982, Toth 2002), electric- or gas-powered fans (Nelson and Chamberlain 1955, Dietrick 1961, Murphey and Darsie 1962, Clark et al. 1994), or laboratory bench vacuum (Schwartz 1964), help to protect workers from harmful inhalation of dust, insect parts, or pathogens (Hurd 1954, Douglas 1984, Kim and Hong 2007). Powered aspirators also substantially reduce worker fatigue and error, increase speed and efficiency, and minimize harm to living insect samples. Early aspirator designs placed the collecting tube in-line with the suction (e.g., Kunkel 1926), but this required the unit to be taken apart to access samples—a poor design for flying insects. Also, as insects were drawn into the collecting tube they would strike and be forced against the protective mesh at the opposite end, often causing injuries and damage.

These shortcomings were soon resolved with the development of the “pooter”-style aspirator (Poos 1929), where the collecting vial was offset at a right angle to the flow of air such that insects were deposited directly into alcohol (Singer 1964), or into a small cage (Peterson 1953: Plate 166) or vial (Matteson 1965) that could be removed and quickly capped. This last feature was especially attractive for use in bioassay operations if the removable vial was in fact the bioassay chamber. Using an innovative bent pick-up tube, Chao and Peterson (1952) designed a T-shaped aspirator that efficiently deposited insects into removable bioassay vials. Ristich and Lockard (1953) quickly followed with a set of quick-change adapters that would permit the T-aspirator to be used with a range of collecting vial sizes.

Recent work using fine insect forceps to transfer anesthetized adult female mosquitoes from colonies to bioassay chambers (Britch et al. 2009, 2010) highlighted the need for an improved aspirator design. Having observed damage to mosquitoes after transfer, and having found that forceps transfer was slow and not keeping up with the period of anesthesia, we specifically designed an aspirator to rapidly and gently pick up and deposit females directly into bioassay tubes. We built upon Chao and Peterson’s (1952) T-aspirator model to develop an aspirator gun (the “Wynn Gun”; Fig. 1) with modern, easily procured materials, and additional innovations to overcome challenges imposed by the mosquito body plan.

Mosquitoes are soft bodied with fine wings and scales that are subject to sticky electrostatic forces. Mosquito bodies are also sensitive to air flow because their mass is diffused over air-catching structures such as long legs, antennae, wings, and proboscis. In initial trials with a basic T-aspirator, we learned that electrostatic adhesion frequently prevented mosquitoes from completing the path to the bottom of the bioassay vial. We also learned that turbulence in the bioassay tube could cause some mosquitoes to flush straight through the device and out into the vacuum motor. As a result we added an antistatic grounding system to the pick-up tube and an airflow baffle to the rear of the expansion chamber within the main body assembly (Fig. 1).

The main body of the Wynn Gun is impact-resistant, rigid polyvinyl chloride (PVC) that can be easily modified with couplers to hold a range of bioassay collection tubes. Similarly, the pick-up tube can be replaced with a range of nozzle diameters to accommodate a range of insect sizes. The separate vacuum blower can be changed to produce a range of suction forces, and the air exhaust can be filtered to protect air quality in the lab. The Wynn Gun was used to aspirate *Culex quinquefasciatus* Say mosquitoes (1995 USDA

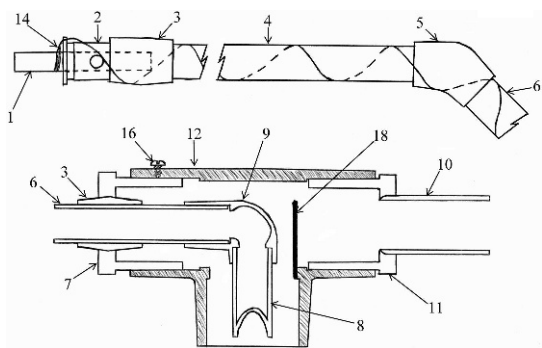


Fig. 1. Cross-sectional detail of Wynn Gun aspirator body and pick-up tube. Numbers are keyed to parts listed in Table 1.

strain, Gainesville, FL) at approximately 60 transfers/min for 360 bioassays of 10 females each in Britch et al. (2011), with a mean of 1% mortality in controls, demonstrating that both the device and the bioassay system are safe for adult mosquito transfer.

Identification of parts and supplies (in Table 1) is keyed to part numbers in the Wynn Gun aspirator with pick-up tube (Fig. 1) and with bioassay tubes and vacuum hose (Fig. 2). Construction of

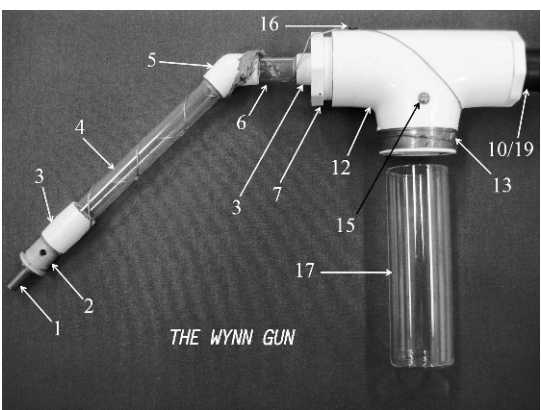


Fig. 2. Exterior photograph of Wynn Gun aspirator with bioassay tube and vacuum hose attached. Numbers are keyed to parts listed in Table 1.

the Wynn Gun is divided into 4 main components for simplicity and efficient assembly. These components are: the pick-up tube, the main body assembly, the electrostatic grounding system, and the independent vacuum source. The pick-up tube component is constructed first. It is made up of brass and acrylic tubing and allows the operator to collect individual insects from a surface. Thin-

Table 1. Parts list of Wynn Gun aspirator assembly as keyed to part numbers in Figs. 1 and 2.

Part no.	Product/part <sup>1</sup>	Dimensions <sup>2</sup>	Quantity	Source <sup>3</sup>
1	Tubing, thin-walled brass	8-mm OD	2.7 in. (6.75 cm)	H, O
2	Fisherbrand rubber pipette bulb <sup>4</sup>	¼-in. ID, 3-ml capacity; 1 ⅝ in. long	1	S, O
3	Straight coupling, CPVC <sup>5</sup>	S/S, ½ in. (½ in.)	2	H, O
4	Tubing, clear acrylic	½-in. ID, ⅝-in. OD	5.5 in. (13.75 cm)	H, O
5	L-coupling, CPVC 45° angle	S/S, ½ in. (½ in.)	1	H, O
6	Tubing, clear acrylic	½-in. ID, ⅝-in. OD	4.15 in. (10.37 cm)	H, O
7	Reducer bushing <sup>6</sup>	Sch. 40, 1 ¼ in. × ½ in.	1	H, O
8	Tubing, clear acrylic <sup>7</sup>	½-in. ID, ⅝-in. OD	1.75 in. (4.37 cm)	H, O
9	L-coupling, CPVC 90° angle <sup>8</sup>	S/S, ½ in. (½ in.)	1	H, O
10	Pipe, PVC thin-walled	1 in.	2 in. (5 cm)	H, O
11	Reducer bushing <sup>9</sup>	Sch. 40, 1 ¼ in. × ¾ in.	1	H, O
12	T-coupling, PVC <sup>10</sup>	Sch. 40, S/S, 1 ¼ in. × 1 ¼ in. × 1 ¼ in.	1	H, O
13	Copper tape, adhesive back	½ in. wide	12 in. (0.3 m)	H, O
14	Wire, noninsulated	24 AWG stranded	24 in. (0.6 m)	H, O
15	Round head machine screw	#6-32 × 8 mm	2	H, O
16	Round head machine screw	#6-32 × 5 mm	2	H, O
17	Test tube (Corning-Pyrex® 9850-40)	40 mm × 130 mm	1	S, O
18	Cardstock with HD packing tape on a side	2 in. × 2 in. square	1	H, O
19	Flexible hose	1 in.	4 ft (1.2 m)	H, O
20	Pipe coupling <sup>11</sup>	S/S, Sch. 40, 1 ¼ in.	1	H, O
21	Velcro® self-grabbing strap	½ in. × 8 in.	1 strip	H, O

<sup>1</sup> CPVC, chlorinated polyvinyl chloride; PVC, polyvinyl chloride; HD, heavy duty.  
<sup>2</sup> OD, outer diam; ID, inner diam; S, slip; Sch., schedule; AWG, American wire gauge.  
<sup>3</sup> H, hardware store; S, scientific supply; O, online.  
<sup>4</sup> Rounded end of bulb removed and 2 opposing ¼-in. (6.2-mm) holes drilled halfway up sides.  
<sup>5</sup> Inside stop flange removed from one coupling.  
<sup>6</sup> Modified to accept the coupling from part no. 3.  
<sup>7</sup> ½-in. (12.5-mm) “V” cut from the bottom.  
<sup>8</sup> ¼ in. (6.2 mm) of the downward end removed.  
<sup>9</sup> Modified to accept thin-walled PVC pipe.  
<sup>10</sup> Mouth of vertical tube of “T” honed out to accommodate thickness of Velcro-strap gasket.  
<sup>11</sup> Used as a stand to hold completed device.

walled brass tubing (Table 1: part no. 1) is inserted into a rubber pipette bulb (Table 1: part no. 2) with the rounded end removed and 2 opposing  $\frac{1}{4}$ -in. (6.2 mm) holes drilled halfway up the sides of the bulb. The drilled holes reduce eddies in the pipette bulb and smooth the flow of air through the pick-up tube assembly. The cut section of the pipette bulb is inserted up to the stop flange in a straight coupling (Table 1: part no. 3). A stop flange is a raised portion within PVC fittings that prevents joined parts from sliding through. A length of clear acrylic tubing (Table 1: part no. 4) is inserted up to the stop flange from the opposite end of the same straight coupling. The other end of the clear acrylic tubing is attached to an L-coupling (Table 1: part no. 5), which is then attached to a 2nd length of clear acrylic tubing (Table 1: part no. 6). This 2nd clear acrylic tube is then inserted completely through another straight coupling (Table 1: part no. 3) that has had the stop flange ground off so that  $\sim 1\frac{3}{4}$  in. (43.7 mm) of the tube is protruding through the coupling. The coupling and acrylic tubing assembly is inserted into a reducer bushing (Table 1: part no. 7) that has been bored out to accept the outside diameter of the coupling. An L-coupling (Table 1: part no. 9) with  $\frac{1}{4}$  in. (6.2 mm) of the downward end removed is attached to the protruding end of the acrylic tubing. Another length of clear acrylic tubing (Table 1: part no. 8) is attached to the trimmed end of the L-coupling, and a  $\frac{1}{2}$ -in. (12.5-mm) "V" is cut from the opposite end of the tubing. The entire series of fittings in the pick-up tube assembly should be cemented using PVC/chlorinated polyvinyl chloride (CPVC) adhesive (e.g., All-Purpose PVC/CPVC cement; product no. 30818; Oatley, Cleveland, OH), except for the proximal joint in the  $45^\circ$  L-coupling (Table 1: part no. 5), which should be allowed to rotate to set a working angle comfortable for the operator, and the last length of clear acrylic tubing (Table 1: part no. 8), which should be attached and cemented after the tube assembly has been affixed to the main body assembly.

The main body assembly is attached to both the vacuum source and the pick-up tube. The electrostatic grounding system cannot be completed and the pick-up tube cannot be attached and cemented in place without the main body assembly. Thin-walled PVC pipe (Table 1: part no. 10) is cut and cemented to a reducer bushing (Table 1: part no. 11) that has been bored out to accept the outside diameter of the pipe. The reducer bushing is attached to one of the lateral openings of a PVC "T" (Table 1: part no. 12) and secured lightly with pressure from a screw (Table 1: part no. 16) inserted through the upper wall of the "T". A piece of cardstock paper (Table 1: part no. 18) is attached with heavy-duty packing tape to the inside rear surface of the "T" to act as a flow restrictor baffle. This baffle reduces the possibility

of an insect being drawn back out of the glass bioassay tube into the vacuum source. The baffle and the vacuum pressure are optimized by leaving a  $\frac{1}{4}$ -in. (6.2 mm) gap at the top between the cardstock paper and interior upper surface of the "T." The bushing of the pick-up tube assembly is then attached to the other lateral opening of the PVC "T" and also secured with a screw (Table 1: part no. 16). The vertical opening of the "T" (i.e., the handgrip) is slightly bored out to accommodate a length of the loop material side of a Velcro® strap adhered with double-sided sealing tape or room-temperature vulcanization (RTV) adhesive (Table 1: part no. 21; not shown). This cushioned gasket creates a vacuum seal and allows easy insertion and removal of the glass bioassay tubes (Table 1: part no. 17; Pyrex® glass culture tube, 40 mm  $\times$  130 mm, rimless, flat-bottom; part no. 9850-40; Corning, Corning, NY). Screws (Table 1: part no. 15) are inserted into the handgrip at  $1\frac{1}{4}$  in. (31.2 mm) from the opening, as stops for the bioassay tubes (Fig. 2: part no. 15).

The electrostatic grounding system is constructed of grounding (noninsulated) wire, RTV adhesive, and copper tape. The grounding system is constructed to utilize the operator as the bridge between the Wynn Gun and the ground. One end of the grounding wire (Table 1: part no. 14) is wrapped 3 times around the exposed part of the brass tubing (Table 1: part no. 1) and anchored with solder. The grounding wire is then wrapped tightly around the pick-up tube and routed to the handgrip as shown in Figs. 1 and 2. The wire must be tightened flush against all surfaces it contacts and to help prevent unraveling, dots of RTV adhesive (e.g., INSTAbond 1645 RTV silicone; National Stock No. 8040-00-145-0020; ACCRAbond, Inc., Olive Branch, MS) can be applied intermittently. Adhesive copper tape (Table 1: part no. 13) is applied to the handgrip to anchor the other end of the grounding wire and provide a good contact to the operator and completing the circuit to the ground.

Finally, the vacuum source is fitted to the system. The thin-walled PVC tube projecting from the rear of the gun (Table 1: part no. 10) is attached via flexible air hose (Table 1: part no. 19) to the intake aperture of an electric blower fan (e.g., a 60-ft<sup>3</sup>/min Dayton blower; part no. 4C441; Grainger Industrial Supply, Lake Forest, IL). For aspirating mosquitoes, the vacuum source should be adjusted to produce an optimal airflow of 1.5 cm<sup>3</sup>/sec through the tip of the pick-up tube. Proper airflow should be checked with an anemometer. A pipe coupling (Table 1: part no. 20; not shown) can be fastened to the lab bench to serve as a stand for the Wynn Gun with bioassay tube attached.

The Wynn Gun is a novel modification of established aspirator designs that allows delicate test organisms to be added rapidly and efficiently

to bioassays, with low mortality rates. Its construction recognizes insect diversity and allows for parts and even entire components to be exchanged to better accommodate operational needs. Future variations on this design include the development of a field-portable Wynn Gun.

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